CHAPTER 3
MATERIALS & METHODS

The study on various aspects and objectives related to the present research work was carried out during the year 2009-2014 at Department of Food Processing Technology, A. D. Patel Institute of Technology, New Vidya Nagar, Anand, College of Food Processing Technology & Bio Energy, AAU, Anand and SMC College of Dairy Science, Anand.

In the present investigation, rennet casein as a protein source obtain from Modern Dairies, Karnal and Vegetable oil from the local market was used to develop the formulation and process technology of partial dairy Mozzarella cheese analogue(MCA). Optimized MCA and Natural cheese were analyzed for their proximate composition, functional properties and sensory evaluation. This chapter deals with the description of materials, experimental set ups, analytical techniques and processing techniques used in various trials.

3.1 MATERIALS
3.1.1 RAW MATERIALS

3.1.1.1 Fat Source

Rice Bran Oil in combination with Safflower Seed oil (Saffola Gold Brand) obtained from local market having 48% MUFA, 32% PUFA and added antioxidants is used as fat source in preparation of cholesterol free cheese.

3.1.1.2 Protein source

The rennet casein was procured from M/s Modern dairies, Karnal, Haryana which was the protein source used in MCA preparation. It was dry powdered rennet casein having 90mesh size and min 80.0 % protein on dry matter.

3.1.1.3 Acidifying agent

The acidifying agents used were lactic acid (C$_3$H$_6$O$_3$) LR grade (M/s. S.D. Fine Chemicals Ltd., Mumbai) and citric acid (C$_6$H$_8$O$_7$), Anhydrous LR grade (M/s. S.D. Fine Chemicals Ltd., Mumbai). Lactic and citric acids were used for adjusting the pH of Mozzarella cheese analogue.
3.1.1.4 Salt
‘Tata’ brand vacuum-evaporated salt manufactured by M/s. Tata Chemicals Ltd., Mumbai was obtained from local market and used for salting of Mozzarella cheese analogue.

3.1.1.5 Emulsifying salts
Tri-sodium citrate, dihydrate of LR grade, di-sodium hydrogen orthophosphate, dihydrate of AR grade and sodium hexa-meta phosphate of LR grade (M/s. S. D. Fine chemicals Ltd., Boisar) and Joha C9 (M/s Food and Pharma Specialities, Noida) were tried out as emulsifying salts in the preparation of MCA.

3.1.1.6 Binder
Modified Starch obtained from M/s Shree Additives was used as binder.

3.1.1.7 Emulsifier
Mono-Di Glyceride (Rikemal P 200s) from Rikevita (Malaysia) obtained from M/S Brentag India Ltd. and Soluwat brand Tween 80 Emulsifier obtained from M/s Shree additives were used as an emulsifier in Mozzarella cheese analogue.

3.1.1.8 Flavor
Butter buds (Aged cheddar), Butter buds (Highly concentrated) obtained from Duke Thompson’s India Private ltd, Indore and Cheese Flavor (liquid) (Nature Identical) obtained from M/s. Mane India Limited was used as flavoring agents.

3.1.2 OTHER MATERIAL

3.1.2.1 Pizza base
Fresh partial pre-baked pizzas were obtained from local bakery at Vidya Nagar.

3.1.2.2 Natural Mozzarella cheese
Natural Mozzarella cheese-Amul Brand was obtained from Amul Shop at VV Nagar.

3.1.2.3 Chemicals and Reagents
Chemicals used in the analysis were of analytical grade from Merck, S.D. Fine and Loba Chem companies.

3.2 METHODS

3.2.1 METHODOLOGY OF MANUFACTURE OF MOZZARELLA CHEESE ANALOGUE
Firstly all the selected ingredients were weighed accurately as per the calculation based on the formulation decided for the Mozzarella cheese analogue. Planetary mixer
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(M/s. Karnavati Engineering Limited, Mehsana Rimek (KALWEKA) Model No. VDM 4 SP) was used for mixing and preparing base for the Mozarella cheese Analogue (Figure 3.1).

For phases I to VII, 500 g of cheese analogue was prepared at a time for each treatment under study. However, for phase VIII, 1 kg of MCA was prepared for each treatment.

The sequential steps followed in preparation of MCA are as under:

1. Part of the calculated amount of water was kept aside for preparing the acid solution (acid:water; 1:10 w/v). The rest of the water (heated at 83 °C) was poured in the Stainless steel bowl of planetary mixer. Temperature was maintained as the bowl was insulated.

2. Blend of emulsifying salts was added to the water and dissolved at 40 rpm for 1 min.

3. A dry blend (2/3) of rennet casein, modified starch, common salt and cheese flavoring in 3 installments was added slowly over the emulsifying salt solution keeping the beater speed at 60 rpm and blending for 1.5 min after every installment.

4. Previously heated vegetable oil (2/3) (Blend of Rice bran and safflower oil (80:20)) (65 °C) was added to the dough in 3 installments after every part addition of rennet casein blend.

5. Half of the acid solution (made in step 1) was added to the dough prepare while the beater was in motion at speed 100 rpm). The dough was manually scraped with a stainless steel ladle intermittently after stopping the beater.

6. Remaining 1/3 rennet casein blend and vegetable oil was added in 2 installment and mixed at beater speed of 60 rpm and blended for 1.5 min after addition of every installment.

7. The hydrated and emulsified casein-starch-oil dough was taken out from bowl of the Mixer and transferred to a stainless steel “karahi”-vessel. The dough was heated gently over fire with continuous mixing.

8. Remaining half of the acid solution was added to the paste formed, throughout uniformly to prevent localized effect, on rennet casein-starch- vegetable oil dough.

9. The heating was continued till the final temperature of cheese mass reached to 80-82 °C (in about 4-5 min). At this stage, curd fusion took place resulting in a hot plastic and stringy cheese mass which stretched to form strands of cheese mass. Peptization of casein takes place at this stage resulting into hot plastic mass which stretched to form strands of cheese mass like natural Mozarella cheese.
10. The hot plastic mass was removed in a stainless steel dish, moulded in to ball form manually, covered with large vessel and allowed to cool naturally to room temperature.

11. The cooled and congealed cheese mass was then packed in polyethylene bag (45 µm) and sealed using a hand sealing machine.

12. The cheese analogue was then transferred to a refrigerator maintained at 7±1 °C for overnight cooling.

13. Next day MCA was analyzed for chemical analysis, backing quality and sensory evaluation on pizza.

*Fig 3.1 Stringy Cheese Mass after final processing Stage*
3.3 FORMULATION AND STANDARDIZATION OF THE RECIPE FOR MOZZARELLA CHEESE ANALOGUE

The experiment involving process development and standardization of the recipe for MCA was carried out in 6 phases.

I. Development of formulation for Mozzarella cheese Analogue.
II. Selection of type of Acid
III. Selection of type of emulsifying salts
IV. Optimizing the proportion of two emulsifying salt.
V. Validating the need of starch in the formulation.
VI. Optimizing the level of rennet casein, rate of addition of emulsifying salt and rate of addition of acid using RSM

PHASE I

3.3.1 DEVELOPMENT OF A FORMULATUION FOR MOZZARELLE CHEESE ANALOGUE

In this part preliminary trials were conducted for screening of several ingredients for manufacture of Mozzarella cheese analogue. As the aim was to produce cholesterol free cheese analogue Rice bran oil in combination with safflower oil was used as fat source. Rennet casein was chosen as protein source to get desired emulsification of fat, good melting and stretchability in the MCA. Potable water was used to get the desired moisture content. Aged cheddar and highly concentrated butter buds and Nature Identical cheese flavor was used as flavoring agent to give dairy flavor as milk fat is not used in the formulation. Other functional ingredients used were binder (modified starch), emulsifier (Mono-di glyceride and tween-80), acid (lactic and citric) and emulsifying salt (Salts of citrates and phosphates).

3.3.1.1 Vegetable oil

The level of oil incorporated was based on obtaining the Fat-on-dry matter (FDM) content confirming to the FSSA standards for Pizza cheese (i.e. minimum 35.0 % FDM).

3.3.1.2. Rennet casein

The rate of rennet casein was initially chosen at 24% w/w in the formulation of MCA.

3.3.1.3 Binder

Modified Starch was used at 3.0 per cent on w/w in the formulation to serve as binder and possibly improve the body-texture, flavor by masking the casein flavor and cost effectiveness as cheaper than casein of the MCA.

3.3.1.4 Emulsifying Salt

In the preliminary trials, Tri sodium citrate (TSC) alone, its combination with disodium hydrogen orthophosphate (DSP) and Sodium Hexa Meta Phosphate (SHMP) was tried out. TSC alone and its combination with DSP did not give desired result. A combination of TSC and SHMP (in proportion of 30:70) @ 3.0 percent yielded cheese analogue having required functional properties and hence was selected for further study.
3.3.1.5 **pH regulator**

Lactic acid and citric acid were evaluated as pH regulator at levels of 0.5 % by weight in the formulation. Use of lactic acid and citric acid yielded product having pH of 5.74 and 5.71 respectively.

3.3.1.6 **Emulsifier**

As vegetable oil without hydrogenation is used as a fat source it was decided to incorporate emulsifier @ 0.2 % in the formulation. Two emulsifiers namely Mono and di glyceride (MDG) and Tween 80 separately used @ 0.2 per cent w/w in the formulation but none of this helped in improving emulsification and MCA prepared was not of desired quality. So in the later study it was discontinued.

3.3.1.7 **Salt**

To enhance the taste of MCA common salt was used at level of 0.9, 1.0 and 1.1 w/w in the formulation. Preliminary trial indicated that a level of 1.0 percent gave the best sensory characteristics to the MCA.

**PHASE II**

3.3.2 **SELECTION OF ACID**

Acidulants play a wide variety of roles in the manufacture of dairy foods. They function in preservation, consistency/texture development and contribution to flavour in processed cheese, cheese foods and cheese spreads; flavour enhancement in cultured and imitation dairy products such as sour cream and related analogues. They act as faster-acting and more predictable replacements for acids produced by bacterial fermentations in direct-set cheeses (Meyer, 1973; Dziezak, 1990).

Two type of acid i.e. lactic and citric were tried out for pH adjustment. Based on preliminary trials it was decided to use lactic acid and citric acid @ 0.50 % by weight in the formulation of MCA to obtain the desired sensory and functional properties. The experiment was replicated seven times.

**PHASE III**

3.3.3 **SELECTION OF TYPE OF EMULSIFYING SALTS**

Emulsifying salt is a major ingredient in analogue/process cheese manufacture. For the production of cheese analogue/processed cheese the most commonly used emulsifying salts are citrates and phosphates. Emulsifying salts participate in an ion-
exchange process by exchanging the calcium bound to casein with sodium, thus improving the emulsifying ability of the proteins with the aid of heat and shear.

Based on the preliminary trials using trisodium citrate (TSC) alone, its admixtures with disodium hydrogen orthophosphate(DSP), sodium hexameta phosphate(SHMP) or trisodium phosphate (TSP), and JOHA C9 alone it was observed that JOHA C9 alone or TSC in combination with SHMP (i.e. TSC:TSP; 30:70) gave better results. Hence, it was essential to find out which out of them (i.e. JOHA C9 and TSC+SHMP) was more suitable for preparation of MCA, when used at level 3.0 per cent level. The experiment was replicated Seven times.

**PHASE IV**

### 3.3.4 OPTIMIZING THE PROPORTION OF TWO EMULSIFYING SALT

In preliminary trial Tri- sodium citrate alone and its combination with di-sodium hydrogen orthophosphate (TSC:DSP 1:2) and (TSC:DSP 2:1) and Sodium hexameta phosphate (TSC:SHMP 1:2) were tried out. TSC when used alone and with combination to DSP did not yield MCA with desired properties. TSC when used in combination with SHMP yield desired product. Hence it was essential to determine proportion of TSC:SHMP that would be highly suited for the functional and sensory properties of cheese analogue. Two proportions of TSC and SHMP were tried i.e. 1:2 and 1:3 both proportion used @ 3.0% by weight in the formulation.

The experiment was replicated seven times.

**PHASE V**

### 3.3.5 VALIDATING THE NEED FOR STARCH IN THE FORMULATION

Starchy material like maltodextrin, pre-gelatinized starches and modified starches has been utilized by several researchers as binder in manufacture of cheese analogues (Jana and Upadhyay, 2001; Mounsey and O’ Riordan, 2001, 2008a,b). It was presumed that use of modified starch (MS) would help in contributing to TS (reducing the water content), which may contribute to better shredding properties. Moreover, modified starch is a cheaper source of solids than rennet casein. Based on preliminary trials, it was found that incorporation of modified starch at 3.0 percent rate was beneficial. Hence, the study was conducted to verify whether incorporation of modified starch would be desirable or otherwise in preparation of cheese analogue. Hence, cheese analogues were
made with 0 and 3.0 per cent level of modified starch the water in the latter formulation was adjusted accordingly.
The experiment was replicated seven times.

PHASE VI

3.3.6 OPTIMIZING LEVEL OF RENNET CASEIN, RATE OF ADDITION OF EMULSIFYING SALT AND RATE OF ADDITION OF ACID USING RESPONSE SURFACE METHODOLOGY (RSM).

In this phase rate of addition of rennet casein, emulsifying salt and acid was optimized using design expert program of the STATE-EASE software. Other ingredients are used at rate according to selection made in Phase I to V.

PHASE VII

3.3.7 COMPARING MCA WITH NATURAL MOZZARELLA CHEESE

In this phase MCA prepared using the standardized formulation was compared against a commercially available Natural (Milk based) Mozzarella cheese (NMC). The MCA and Natural cheese were subjected to chemical analysis, baking qualities, microbiological as well as for their sensory analysis (i.e. actual baking trial on pizza pie). Seven replications were undertaken for this part of the study.

PHASE VIII

3.3.8 ASSESSING THE COST EFFECTIVENESS OF MOZZARELLA CHEESE ANALOGUE VIS A VIS NATURAL MOZZARELLA CHEESE.

To find out the commercial/economic viability of MCA production vis-à-vis natural Mozzarella cheese, their costing was carried out making necessary appropriate assumptions, wherever necessary. Costing was carried out for 100 kg product in both cases. The basis of cost calculations, the assumptions made and the calculated cost are given in Appendices.

3.4 COMPOSITIONAL ANALYSIS

3.4.1 RENNET CASEIN

Rennet casein was analyzed for moisture, fat, nitrogen and total ash according to the procedures laid down by BIS (IS: 1167, 1965).

3.4.1.1 Determination of Moisture
Moisture of the casein samples were determined by the standard procedure using Mojonnier Milk Tester, Model-D (Milk Industry Foundation, 1959). About 1.0 g of sample was accurately weighed in a clean and dried Mojonnier moisture dish. To this, 2-3 ml of hot distilled water was added to make a paste, which was then spread over the flat bottom of the dish. The dish was then placed on a hot plate at 180˚C for drying till the residue attained brown colour. The dish was then transferred to the Mojonnier vacuum (50 cm of vacuum) oven at 110˚C, and held there for at least 25 min. Finally, the dish was transferred to an adjacent desiccator for cooling, followed by weighing of the dishes in precision weighing balance (Precisa Instruments Ltd., Switzerland). The heating, cooling and weighing was continued until the difference between two subsequent weighing did not differ more than 5 mg. The per cent moisture in sample was calculated using the formula.

\[
\text{Moisture(\%)} = \frac{\text{Loss in weight of sample}}{\text{Weight of sample taken}} \times 100
\]

3.4.1.2 Determination of Fat

Five grams of casein was added to 10 ml of concentrated hydrochloric acid contained in 100 ml beaker. An additional 10 ml of acid was used to wash down particles adhering to the beaker. The contents were heated over a burner and boiled for 10 min. After cooling, the contents were quantitatively transferred to a Mojonnier extraction flask using 10 ml of acid as wash liquid. Next, 25 ml each of ethyl ether and petroleum ether were sequentially added first to the beaker and then transferred to extraction flask. The flask was shaken vigorously for 1 min each time. The flask was centrifuged at 6000 rpm and ether solution was decanted into a previously weighed dry metal dish. The fat remaining in the flask was extracted by repeating the above procedure twice, using 15 ml each of above mentioned solvents. The solvent was then evaporated on a hot-plate maintained at 105˚C. The fat contained in the dish was dried in an oven at 105˚C to a constant weight and expressed as fat per cent.

3.4.1.3 Determination of Total Nitrogen

The nitrogen content was determined by the Kjeldahl method using KEL-Plus System (Model No.: KES 12L, M/s. Pelican Instruments, Chennai) machine (Fig 3.2 A&B) (Jayaraman, 1981) Facility Available at College of FPT & BE. The value of nitrogen was multiplied by a factor of 6.38 to arrive at the protein content.

Dried casein sample was accurately weighed (0.2 g) and taken in digestion tubes. To the tubes, 10 ml of concentrated sulphuric acid (36N) and a spatula full (about 2 g) of
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digestion mixture (Potassium sulphate: copper sulphate: selenium dioxide in 10:0.2:0.2 proportion) was added in the digestion flask. Six digestion tubes were simultaneously placed in a heating mantle of KEL-plus machine maintained at 350°C and kept till the contents became light green in color (requiring ~1 h). After cooling, the digestion tube was fitted in the Kjel–plus distillation unit wherein about 20 ml of 40.0 percent sodium hydroxide solution was introduced giving a brown colour. A conical flask containing 25 ml of saturated boric acid solution and a few drops of mixed indicator (methylene blue and methyl red) was kept at the delivery end of the condenser tube. The steam generated in the boiler of the machine heated and boiled the contents in digestion tube. Ammonia was fixed in boric acid as ammonium borate. The distillation was completed in 3-4 minutes.

The distillate was titrated against 0.05 N sulphuric acid, till the colour changed from green to pink. A reagent blank was simultaneously run using all the above chemicals except the samples and the reading was subtracted from the sample reading. The percent total nitrogen was calculated using following formula:

\[
\text{Total Nitrogen(\%) = 0.07 \times \frac{R - B}{W}}
\]

Where,

\( W \) = Weight of Sample
\( R \) = Volume of 0.05 N H\(_2\)SO\(_4\) used in titrating the sample
\( B \) = Volume of 0.05 N H\(_2\)SO\(_4\) used in titrating the Blank

The percent total protein content was calculated by multiplying a factor of 6.38 with total nitrogen obtained as above.

3.4.1.4 Determination of total ash

The ash content of the casein samples was determined using 3.0 g of sample and following the standard BIS method for milk (BIS, 1961b).

About 2-3 g of sample was accordingly weighed in a silica crucible. The sample in crucible was heated on naked-flame till it became ash. The sample was then transferred to a muffle furnace (Narang scientific works Pvt. Ltd., 101/23673, New Delhi) and held for 3.5 h at a temperature of 550±2°C. After cooling in a desiccator, the crucible was weighed. The process was repeated till constant weight was achieved. The total ash content was calculated as follows:

\[
\text{Total ash(\%) = \frac{(W_2 - W)}{(W_1 - W)} \times 100}
\]
Where,
\[ W = \text{Weight (in g) of the empty crucible} \]
\[ W_1 = \text{Weight (in g) of the crucible with sample} \]
\[ W_2 = \text{Weight (in g) of the crucible with ash} \]

3.4.1.5 Determination of pH
The pH of the casein was determined by the method suggested by Southward (1985). Casein (5 g) was dissolved in 30 ml of distilled water and made into a paste. This paste was subjected to pH determination using a digital pen pH meter, Model-PH 600 (M/s. Mill wake, 2008).
3.4.2 **VEGETABLE OIL**

3.4.2.1 **Moisture**

Ten gram of fat sample was taken in a clean dry aluminum dish. The dish was kept in hot air oven (Oven universal-143) maintained at 105 ± 1 °C for about 1 h. The dish was removed from the oven and cooled to room temperature in a desiccator. The dish was then weighed. The process of heating, cooling and weighing were repeated every half an hour until the difference between two successive readings did not exceed 1.0 mg. The moisture content was calculated as:

\[
\text{Moisture(\%)} = \frac{\text{Loss in weight of sample}}{\text{Weight of sample taken}} \times 100
\]

3.4.2.2 **Free fatty acids**

The free fatty acid (FFA) content was determined by BIS method (BIS-1975). Ten gram of sample was taken in a conical flask (50 ml). In another conical flask, 50 ml of ethyl alcohol (95.0 per cent, v/v) was boiled and neutralized with 0.1 N sodium...
hydroxide. This neutralized alcohol was poured on the sample contained in the flask and contents were mixed thoroughly. It was brought to boil and while hot, the contents were titrated against 0.1 N sodium hydroxide. The FFA was calculated as:

$$\text{FFA (per cent oleic acid)} = 2.82 \times \frac{T}{W}$$

Where,  
$W$ = weight (g) of sample  
$T$ = ml of 0.1 N sodium hydroxide required for titration.

### 3.4.2.3 Peroxide value

The peroxide value of specialty vegetable fat was determined as per the standard method (BIS, 1975).

One gram of the sample was weighed into a test tube (150 X 25 mm) in liquefied form and to this 1.0 g of powdered potassium iodide and 20 ml of the solvent mixture (glacial acetic acid and chloroform; 2;1) was added. The contents were heated to boiling in a water bath within 30 s and allowed to boil vigorously for 30 s. The opening in the bung was closed with a glass rod as soon as the solvent vapour began to escape. The content was cooled immediately under tap water and then transferred to a conical flask containing 20 ml of 5.0 per cent aqueous solution of potassium iodide. The test tube was washed twice with about 25-30 ml of distilled water. The solution was titrated against 0.002 N sodium thiosulphate solution using starch as an indicator. A blank was also performed.

$$\text{ml of 0.002 N Na}_2\text{S}_2\text{O}_3 \text{ solution/g of sample} = \frac{T}{W}$$

Where,  
$W$ = Weight in g of sample  
$T$ = volume in ml of 0.002 N Na$_2$S$_2$O$_3$ solution required by sample

### 3.4.3 MOZZARELLA CHEESE ANALOGUE & NATURAL MOZZARELLA CHEESE

The natural Mozzarella cheese (NMC) and Mozzarella cheese analogue (MCA) were subjected to analysis for their proximate composition, objective baking qualities and sensory evaluation of cheese as a topping on pizza pie.

#### 3.4.3.1 Sampling

After overnight cooling of the cheese analogues in the refrigerator (7°C), sealed in polyethylene bags, a portion of about 250 g was used for melting test, pizza making.
About 100 g of cheese was minced, mixed well and used for chemical analyses includes total solids, fat, protein, ash, salt (NaCl) and pH.

3.4.3.2 Compositional analyses

3.4.3.2.1 pH

Twenty grams of cheese sample was mixed with 20 ml of distilled water and a fine paste was obtained. The pH readings were taken on a Systronics Digital pH meter, Model-962 P (M/s. Max Electronics, Chandigarh).

3.4.3.2.2 Determination of Total Solids

Total Solids of MCA was determined by the method described in the FSSAI Manual for testing of Milk and Milk products (Ref: IS 2785-1979) as below:

1. Heat the flat bottom metal dish containing 20 g of prepared sand and a stirring rod, in hot air oven for about 1 h. Allow to cool in an efficient desiccators for 30 to 40 min. Weigh accurately 3 g of the prepared sample of MCA into a flat-bottomed dish (with cover) previously dried and weighed containing about 20 g of sand and stirring rod.

2. Saturate the sand by careful addition of a few drops of distilled water, and thoroughly mix the wet sand with the MCA sample by stirring with the glass rod, smoothing out lumps and spreading the mixture over the bottom of the dish.

3. Place the dish on a boiling water bath for 20 to 30 min, then wipe the bottom of the dish. Transfer the dish containing the material, along with glass rod after uncovering in an oven maintained at 102 ± 1 °C for about 4 hr.

4. After 4 hour replace the lid, transfer the covered dish to the desiccator, allow it to cool to room temperature and weigh it accurately and quickly to the nearest 0.1 mg.

5. Heat the uncovered dish and lid in the oven at 102 ± 1 °C for further 1 h, replace the lid, allow the covered dish to cool to room temperature in the desiccator and weigh it. Repeat the process of drying, cooling and weighing until the successive weighing do not differ by more than 0.5 mg. Record the weight.

\[
\text{Moisture (\% by mass)} = \left(\frac{W_1 - W_2}{W_1 - W_3}\right) \times 100
\]

Where,

- \( W_1 \) = Initial mass in g of the dish, lid, glass rod along with sample for analysis before drying
- \( W_2 \) = The final mass in g of the dish, lid, glass rod along with sample for analysis after drying
- \( W_3 \) = Initial mass in g of the dish, lid, glass rod
W\textsubscript{3} = mass in g, of the empty dish with glass rod.
(Ref: IS 10484 -1983 Specification for Paneer)

3.4.3.2.3 Determination of Fat
Weigh accurately 1-2 g of prepared sample in a 100 ml beaker. Add 10 ml of conc. Hydrochloric acid. Heat on a Bunsen burner, stirring continuously with a glass rod, or on a boiling water bath until all solid articles are dissolved. Cool to room temperature. Add 10 ml of ethyl alcohol first to the beaker and later transfer the contents to the Mojonnier fat extraction flask or the Rohrig tube. Proceed as in determination of milk fat by acid digestion.

3.4.3.2.4 Protein
Total nitrogen of cheese was determined by semi-microKjeldahl method (Jayaraman, 1981), using KEl-plus digestion system (Model-KES 12L, M/s.Pelican Instruments, Chennai) and Kjel-plus Fully automatic distillation system (Model-CLASSIC DX, M/s.Pelican Instruments, Chennai). The method is same as described in section 3.4.1.3, except that 0.3-0.4 g of cheese was taken for analysis.

3.4.2.3.5 Ash
The ash content of the cheese samples was determined using the method described in section 3.4.1.4, taking cheese instead of casein.

3.4.2.3.6 Salt
The salt (sodium chloride) content of cheese samples was determined by modified Volhard method using nitrobenzene, according to the procedure outlined by Kosikowski (1982).

3.0 g of cheese sample was weighed in a 250 ml conical flask and to this 25 ml of 0.1 N silver nitrate, 10 ml of halogen-free concentrated nitric acid and 50 ml of distilled water was added. The mixture was heated to boiling and to that 15 ml of fresh 5.0 per cent potassium permanganate solution was added in three, 5 ml portions. The digested yellowish solution was cooled to ambient temperature and then 2 ml each of nitrobenzene and saturated ferric ammonium sulphate were added. The contents were titrated with 0.1 N potassium thiocyanate to a brick-red end point. The titration value was converted to equivalent sodium chloride and expressed as percent salt in cheese. A reagent blank was simultaneously run with 3.0 g of sucrose to nullify the effect of excess permanganate.

The chemical analyses of milk, casein, natural cheese / cheese analogue for relevant parameters were done in duplicate for each sample.
3.4.3.3 Evaluation of baking properties of MCA/NMC

3.4.3.3.1 Shredability

The shredding properties of natural/analogue Mozzarella cheese is of much significance since it is invariably used as a topping on pizza pie. For the same, the cheese is first shredded and then applied on pizza pie. Nowadays, more convenient “packaged shredded cheese” is also available for direct application on pizza base.

The cheese (MCA and NMC) was shred through a clean stainless steel manual shredder having a pore size of about 1 mm. The ‘shredability’ of cheese was subjectively assessed. It has been our observation that if the cheese can be shred through a shredder with minimum of effort and the shreds obtained are ‘thin and long’ without having tendency to mat with each other, it is considered desirable for pizza application. Hence, cheese shred having the above mentioned characteristics has been referred to as “Excellent” shredding quality. The cheese requiring some effort to shred and if the shreds formed were ‘short and thick’, and having tendency to mat, such cheese were referred to as possessing “Good” shredding quality. The shreds having characteristics in between the above two types has been characterized as “Very good” shredding property.

3.4.3.3.2 Meltability

Meltability of processed cheese analogs was measured using a modified Schreiber test [Mleko & Foegeding, 2001]. Specimens (4.8 mm thick, 41 mm in diameter) were placed in a microwave oven (IFB Make) and heated for 30s. The specimens were then removed and cooled. Their expansion was measured along 6 lines marked on a concentric set of circles as described by Kosikowski[1977]. Schreiber meltability (arbitrary scale of 0-10 units) was given as mean of 6 readings for each of 7 replications. Meltability was defined as the percent increase in diameter of the melted cheese compared with the original diameter.

3.4.3.3.3 Stretch Test

Stretchability of the mozzarella cheese analogs was analyzed by the method described by Guinee and O’Callaghan (1996). The sample was shredded on pizza base (Dia 17 cm, height 1.5 cm using a load of 25 g shredded cheese/mm². The pizza base was placed in a thermostatically controlled electric oven at 250°C for 4 min to simulate the way cheese was heated in practice. The stretchability test was performed at room temperature (25°C) within 1 min after the cheese had been removed from the oven. The heated sample was gripped with a pair of self tightening grips (from Lloyd TA LS, Lloyd Instruments Ltd., UK). The stretching speed was set at 0.075 m/s and the sample
was stretched until the extended string of the melted cheese mass was broken completely. The distance of complete strand breakage was recorded as stretchability (mm).

Fig 3.4 Representation of Melting est

3.4.3.4 PIZZA

3.4.3.4.1 Topping of pizza with cheese

The suitability of the experimental cheese, both natural and analogue, for pizza making was evaluated by actual baking trials. The freshly prepared pizza base (~17 cm diameter, 1.5 cm thick) was procured from a local bakery from Vidya Nagar, Anand. Seventy grams of freshly shredded cheese was topped on each pizza base. As the study involved evaluation of the quality of cheese, vegetable filling was not used at all and the topping of pizza with cheese was done at higher levels than is normally used at the restaurants. The topped pizzas were transferred to a forced draft hot air oven maintained at 230°C and kept there until the cheese melted completely. The segmented triangular pieces of pizza, obtained with the help of a pizza cutter, were then served to each judge (total 6 judges) in hot condition for sensory evaluation.

3.4.3.4.2 Sensory evaluation of pizza

Pizza prepared using the experimental cheeses were subjected to sensory evaluation by the panel of seven judges. A 10-point hedonic scale (Appendix–II) was used for scoring attributes such as appearance (considering melting, fat leakage and
browning too), flavour, melting, stringiness, chewiness and finally scoring out of the total score of 50. The judges were well aware of the characteristics of the cheese required for pizza.

![Microwave oven](image)

**Fig 3.5 Microwave oven**

### 3.5 STATISTICAL ANALYSIS

#### 3.5.1 FOR PHASE I to V

The mean value of each attribute under study obtained from duplicate samples of six to seven replications (two treatments) or four replications (three treatments), were subjected to statistical analysis using ‘Completely Randomized Design’ with equal number of observations. The statistical model adopted was as given by Steel and Torrie (1980), which is illustrated as given below:

\[
Y_{ij} = \mu + T_i + E_{ij}
\]

Where, \(Y_{ij}\) = Response due to jth observation in the ith treatment,

\(\mu\) = General mean,

\(T_i\) = Effect of ith treatment, and

\(E_{ij}\) = Error due to jth observation in the ith treatment.

In case of Standard Plate Count, the log transformed values were used. Since coliform and yeast and mould were not detected either in fresh analogue or natural cheeses, such counts were not subjected to statistical analysis.
3.5.2 **STATISTICAL ANALYSIS IN PHASE VI**

Data was analyzed with Daniel’s XL Toolbox version 5.08 using one-way analysis of Variance (ANOVA) with tukey comparison of means values. Difference between mean values with probability $p < 0.05$ were recognized as statistically significant difference.

**Experimental Design.**

RSM was used to generate the experimental designs, statistical analysis and regression model, with the help of design Expert software Version 8 (Statease Inc.). The central composite rotatable design (CCRD) with a quadratic model (Box and Draper, 1987) was employed. Three independent variables namely Rennet casein level, level of Emulsifying salt and acid level were chosen. Each independent variable had 3 levels which were -1, 0, +1. A total of 20 different combinations (including six replicates of the center point each signed the coded value (0) were chosen in random order according to a CCRD configuration for three factors divided in three blocks (Cochran and Cox, 1957). The $\alpha$-values in the design outside the ranges were selected for rotability of the design (Thompson, 1982).

The response function measured were Meltability, Stretchability, and pH of the MCA. These values were related to the coded variables ($x_i$ i=1,2,3) by a second degree polynomial using the equation below:

$$y = ax^2 + bx + c$$

The co-efficient of the polynomial were represented by $b_0$ (constant terms) $b_1, b_2,$ and $b_3$ (linear effects), $b_{11}, b_{22},$ and $b_{33}$ (Quadratic effects) and $b_{12}, b_{13}, b_{23},$ (interacting effects). The analysis of variance (ANOVA) tables were generated and the effect and regression coefficients of individual linear, quadratic and interactions terms were determined. The significance of all terms in the polynomial were judged statistically by computing the F-value and compared with standard significance level of 0.1%, 1.0 % and 5.0%. The regression coefficient was then used to make statistical calculation to generate contour maps from the regression models.